

ON A NEW METHOD OF PRODUCING IMMUNITY FROM CONTAGIOUS DISEASES.

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More than four years ago * one of us, in the study of the subject of insusceptibility to contagious diseases, reached the conclusion that, in those diseases in which one attack protects from the effects of the contagion in the future, the germs of such maladies were only able to multiply in the body of the individual attacked because of a poisonous principle or substance which was produced during the multiplication of those germs. And also that, after being exposed for a certain time to the influence of this poison, the animal bioplasm was no longer sufficiently affected by it to produce that profound depression and modification of the vital activity which alone allowed the growth of the pathogenic germs and the consequent development of the processes of disease. After several series of experiments, made at that time with only negative results, it became necessary to suspend these investigations until points connected with them, and which were then obscure, should be cleared up, and until it should become possible to repeat the experiments under more favorable conditions. Our expectations in regard to this important subject have at last been realized by the results of experiments recently made in the laboratory of the Bureau of Animal Industry.

The bacterium, which we have lately discovered and which we believe to be the cause of swine plague, is killed in liquid cultures by an exposure to 58° C. for about ten minutes.

This method of destroying the bacterium in liquid cultures was resorted to in studying the effects on pigeons of the chemical

* Department of Agriculture, Annual Report, 1881-2, pp. 290-295.

products (ptomaines?) formed by the bacteria in their vegetative state, and which are probably dissolved in the culture liquid. The heated cultures used in these experiments were always tested by inoculating fresh tubes therefrom, and, if no growth followed this inoculation, the death of the microbes was considered established.

It had been previously determined that the subcutaneous injection of .75 cc. ($\frac{3}{16}$ dram) of a liquid culture of the swine plague bacterium containing 1% of peptone was invariably fatal, in the majority of pigeons within 24 hours. One half of this dose was fatal to a few only.

As a preliminary experiment, four pigeons were inoculated December 24, 1885, with a liquid culture that had been heated for 2 hours at 58°-60° C. Three of these (Nos. 10, 8, 9) received subcutaneously .4, .8, and 1.5 cc. of the heated culture, respectively. The fourth (No. 7) received 1.5 cc. of the pure culture liquid, into which no microbes had been introduced. No. 9, the one which had received the largest dose, was evidently sick the next day, but slowly recovered. The others did not show any symptoms of illness.

January 11, the one which had received a hypodermic injection of the simple culture liquid (No. 7), and the one which had received the largest dose of heated virus (No. 9), received subcutaneously about .75 cc. each of a liquid culture five days old, which had been prepared from a potato culture 15 days old. It is probable that this virus was not so strong, therefore, as a more recent culture from the pig would have been. Both pigeons were sick on the following day. No. 7 died seven days after inoculation. The bacterium of swine plague was found abundantly in the pectoral muscle, in the spleen, kidneys, and liver in moderate numbers.* The other pigeon (No. 9) slowly recovered, but had

*In this animal the major part of both pectoral muscles appeared as if they had been boiled; they were whitish, bloodless; the fibres could be easily broken and crushed with the forceps. The muscular tissue surrounding the dead portion was very dark, gorged with blood. The liver was dark in patches; spleen and kidney pale.

lost the use of its legs. It seemed perfectly well when killed, 15 days after inoculation. It was quite fat, the crop filled with food. In the pectorals were found imbedded two elongated masses of dead tissue or sequestra about 2 cm. long and 1 cm. in diameter, entirely separated from the surrounding tissue by a dense, smooth membrane. In this animal the multiplication of the pathogenic bacteria was purely local, the resistance of the tissues being sufficiently powerful to confine, and finally destroy, the bacteria. The sequestra were made up of dead muscular fibre, which was pale and parboiled in appearance. Each was enveloped by a more or less hyaline homogeneous layer. A liquid culture, inoculated with blood from the heart, remained sterile.

This experiment pointed evidently to an immunity obtained from the chemical products of the bacterium of swine plague. To confirm this view another experiment was made.

January 21, three pigeons (Nos. 11, 12, 13) received hypodermically 1.5 cc. of heated culture liquid in which the bacterium of swine plague had multiplied for two weeks, and was then destroyed by exposure to 58°-60° C. for several hours. A fourth pigeon (No. 14) was kept as a check. No. 10, which had received .4 cc. of heated virus Dec. 24, now received a second dose, this time of 1.5 cc. For the following three or four days all were somewhat ill, and remained rather quiet, with feathers slightly ruffled.

January 29, when all seemed well, three of the four (Nos. 10, 11, and 12) received hypodermically another dose of 1.5 cc. of heated culture liquid. The other (No. 13) had been fiercely attacked by its fellows, and its head was so injured that it was thought best not to give it an injection at this time, and it was placed in a spacious coop alone. None of the birds seemed much affected by this dose.

February 6, a final injection was practised upon the four, No. 13 having recovered from the effects of its injuries. The dose was, as before, 1.5 cc. All seemed well a few days later.

February 13, one week after the last injection, these birds were inoculated with strong virus, the quantity injected being .75 cc., which had hitherto proved invariably fatal, with the single exception of the bird that had been previously treated with heated virus. Those inoculated were Nos. 10, 11, 12, 13, which had received the heated virus, also No. 14, the check pigeon, which had not been touched, and No. 8, which had received a small quantity, .8 cc. of heated virus, December 24, over 50 days before.

On the following day the check pigeon (No. 14) was found dead; the one which had received the smaller dose (No. 8) was very ill and died before the next day. The other pigeons were perfectly well. The effect of this dose of strong virus, so remarkable on the unprotected pigeons, was even more evanescent than that of the heated virus in which all life had been destroyed.

There can be no doubt, therefore, from this very positive result, that the pigeons had acquired an immunity through the effect upon the tissues of the chemical products formed by the bacterium in the culture liquid.

A table giving the dates of the injections and the quantity introduced into each animal is given below :

| Pigeons. | 1885. | 1886. | | | | | Total of Heated Virus. | Remarks. |
|------------|------------------|------------------|------------------|------------------|------------------|-----------|---|----------|
| | Dec. 24. | Jan. 21. | Jan. 29. | Feb. 6. | Feb. 13. | | | |
| | Heated Virus. | Heated Virus. | Heated Virus. | Heated Virus. | Strong Virus. | | | |
| No. 8..... | cc. .8 | cc. | cc. | cc. | cc. .75 | cc. .8 | Died in 48 hours after injection of strong virus. | |
| " 10..... | .4 | 1.5 | 1.5 | 1.5 | .75 | 4.9 | Well Feb. 20. | |
| " 11..... | | 1.5 | 1.5 | 1.5 | .75 | 4.5 | Same. | |
| " 12..... | | 1.5 | 1.5 | 1.5 | .75 | 4.5 | Same. | |
| " 13..... | | 1.5 | | 1.5 | .75 | 3.0 | Same. | |
| " 14..... | | | | | .75 | | Died in 24 hours after injection of strong virus. | |

In the birds that died, (Nos. 8 and 14), the pectoral muscles at the place of injection were pale and friable. Necrosis was

already at hand. The internal organs were not macroscopically altered, excepting the spleen of No. 8, which was enlarged and dark. The presence of the bacterium of swine plague in the blood from the heart was demonstrated by liquid cultures, which, inoculated with a minimum quantity of blood, were turbid with this specific microbe on the following day.

The conclusions to be drawn from this experiment we believe are of superlative importance to a correct understanding of the phenomena of contagious diseases, and the methods by which these diseases are to be combated. They probably apply to all bacterial plagues of men and animals in which one attack confers immunity from the effects of that particular virus in the future. These conclusions are :

1. Immunity is the result of the exposure of the bioplasm of the animal body to the chemical products of the growth of the specific microbes which constitute the virus of contagious fevers.
2. These particular chemical products are produced by the growth of the microbes in suitable culture liquids in the laboratory, as well as in the liquids and tissues of the body.
3. Immunity may be produced by introducing into the animal body such chemical products that have been produced in the laboratory.